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Influence of dietary endophyte (*Neotyphodium coenophialum*)-infected tall fescue (*Festuca arundinacea*) seed on fecal shedding of antibiotic resistance-selected *Escherichia coli* O157:H7 in ewes^{1,2}

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ABSTRACT: The objectives were to determine the effects of short-term feeding of a toxic endophyte (*Neotyphodium coenophialum*)-infected tall fescue seed (*Festuca arundinacea*, cultivar 'Kentucky 31') on fecal shedding and intestinal concentrations of *Escherichia coli* O157:H7 and the concentrations of prolactin, cortisol, and NEFA in experimentally inoculated ewes. Twelve ewes (mean BW = 46 ± 2 kg) were fed a diet containing either high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed for 7 d. Each diet consisted of 50% (as-fed basis) tall fescue seed. Ewes were experimentally inoculated with antibiotic resistance-selected *E. coli* O157:H7 on d 1 of the feeding treatment, and fecal shedding of inoculated pathogens was monitored daily on d 2 to 6. On d 7, ewes were weighed and euthanized, and tissues and contents were sampled from the ileum, cecum, and rectum for quantitative enumeration of *E. coli* O157:H7. Urine was collected at euthanization to determine total ergot alkaloid concentrations. Ewes fed HI-E had lower ($P < 0.001$) DMI than did ewes fed LO-E (0.8 and 1.6 ± 0.1 kg/d of DMI for HI-E and LO-E ewes, respectively); consequently, there was a tendency ($P = 0.06$) for HI-E ewes

to lose 0.3 ± 0.4 kg of BW/d and LO-E ewes to gain 0.2 ± 0.4 kg of BW/d during the 7 d. Urinary ergot alkaloids were increased ($P < 0.001$) in ewes fed HI-E (47.8 ± 9.4 ng/mg of creatinine) compared with those fed LO-E (6.2 ± 9.4 ng/mg of creatinine). Prolactin tended ($P = 0.06$) to be decreased in ewes fed HI-E (7.2 ± 7.0 ng/mL) compared with those fed LO-E (27.7 ± 7.0 ng/mL). Fecal shedding of *E. coli* O157:H7 tended ($P = 0.06$) to be increased in HI-E ewes [5.4 cfu (log₁₀)/g of feces] compared with LO-E ewes [4.5 cfu (log₁₀)/g of feces]. The population of *E. coli* O157:H7 in luminal contents from the ileum, cecum, and rectum did not differ ($P > 0.36$) between treatments. Treatment did not influence ($P = 0.30$) the occurrence of *E. coli* O157:H7 in cecal or rectal tissues; however, ileal tissues from HI-E ewes tended ($P = 0.12$) to have an increased incidence of *E. coli* O157:H7. Concentrations of NEFA tended ($P = 0.12$) to be greater in HI-E ewes than in LO-E ewes, whereas cortisol was similar ($P = 0.49$) for HI-E and LO-E ewes. We conclude that short-term feeding of HI-E tall fescue seed may alter the concentrations of prolactin and NEFA, and may increase fecal shedding of *E. coli* O157:H7 in experimentally inoculated ewes.

Key words: dry matter intake, *Escherichia coli* O157:H7, ewe, nonesterified fatty acid, tall fescue

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INTRODUCTION

Ruminants grazing toxic endophyte (*Neotyphodium coenophialum*)-infected tall fescue (*Festuca arundinacea*)

are exposed to numerous ergot alkaloids that cause several stress disorders, collectively characterized as fescue toxicosis (Hoveland et al., 1983; Paterson et al., 1995). Dry matter intake is usually reduced in ruminants consuming toxic tall fescue (Paterson et al., 1995; Parish et al., 2003), and changes in the diet may influ-

¹Names are necessary to report factually on available data; however, the USDA does not guarantee or warrant the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that also may be suitable.

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ence fecal shedding of pathogenic bacteria from ruminants (Callaway et al., 2003). Fecal shedding of *Escherichia coli* was increased when calves were fasted before (Cray et al., 1998) but not after inoculation with *E. coli* (Cray et al., 1998; Harmon et al., 1999).

Consumption of toxic endophyte-infected tall fescue generally decreases circulating concentrations of prolactin (Schillo et al., 1988; Porter and Thompson, 1992); consequently, prolactin is frequently used as a physiological indicator of fescue toxicosis in ruminants (Aiken et al., 2006). Changes in cortisol in response to consumption of toxic endophyte-infected tall fescue have not been consistent and may depend on the duration of exposure (Fiorito et al., 1991; Aldrich et al., 1993a; Browning et al., 1998). Decreased DMI has been associated with increased concentrations of NEFA in cattle (Bossis et al., 1999; Ciccioli et al., 2003).

We hypothesized that consumption of toxic endophyte-infected tall fescue seed before inoculation of ewes with antibiotic resistance-selected *E. coli* O157:H7 would induce dietary stress by reducing DMI and might result in increased fecal shedding of *E. coli* O157:H7. Therefore, the objectives of the current study were to determine the effects of short-term (7-d) feeding of high toxic endophyte-infected (**HI-E**) or low toxic endophyte-infected (**LO-E**) tall fescue seed diets on fecal shedding and intestinal concentrations of antibiotic resistance-selected *E. coli* O157:H7, and serum concentrations of prolactin, cortisol, and NEFA in experimentally inoculated ewes.

MATERIALS AND METHODS

Animals and Experimental Design

The Animal Care and Use Committee of the USDA-ARS, Food and Feed Safety Research Laboratory, approved the care, use, and handling of the experimental animals. Twelve nonlactating hair-type ewes ($n = 6$ each of Katadhin and St. Croix; mean BW = 46 ± 2 kg; mean age = 2.6 ± 1.5 yr) were blocked by BW and breed, and were housed indoors in individual pens at $23.3 \pm 0.5^\circ\text{C}$ and 14 h of light daily for 2 wk. Ewes had not been exposed to endophyte-infected tall fescue the previous 6 mo before initiation of the experiment. Ewes were acclimated to the diet (cracked corn substituted for fescue seed) for 7 d before initiation of the experiment. On d 0 of the experiment, ewes were weighed (nonfasted) and fed a diet containing either HI-E or LO-E tall fescue seed (*F. arundinacea*, cultivar 'Kentucky 31') for 7 d (Table 1; Burke et al., 2006). Nutrient analysis of the feed was completed by the Agricultural Diagnostic Service Laboratory, University of Arkansas. The diets met NRC (1985) recommendations for CP and TDN. Ewes were offered the diet at 3.5% BW, and orts were weighed daily to calculate DMI. Concentrations of ergovaline in fescue seed and in the total diets were determined by HPLC (Moubarak et al., 1993) with a lower detection limit of 25 ng/g.

Table 1. Composition (as-fed basis) and nutrient analysis (DM basis) of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed diets¹ fed for 7 d to experimentally inoculated ewes

Item	HI-E	LO-E
HI-E fescue seed, %	50.0	—
LO-E fescue seed, %	—	50.0
Cottonseed hulls, %	15.0	15.0
Cracked corn, %	11.1	11.1
Corn gluten, %	8.3	8.3
Soyhulls, %	6.4	6.4
Molasses, %	6.6	6.6
Vitamins A, D, and E, %	0.1	0.1
Limestone, %	1.4	1.4
Dicalcium, %	0.9	0.9
Salt, %	0.2	0.2
Nutrient analysis (DM basis)		
CP, %	20.4	19.8
ADF, %	16.2	18.6
NDF, %	28.4	32.9
TDN, %	76.8	74.3
Ergovaline, mg/kg	0.75 ^a	0.11 ^b

^{a,b}Values in a row with no superscript in common differ ($P = 0.001$).

¹Formulated to meet or exceed the NRC (1985) nutrient requirements.

The ewes were individually inoculated via oral gavage with 10 mL of tryptic soy broth (**TSB**; Sigma Chemical Co., St. Louis, MO) containing the antibiotic resistance-selected *E. coli* O157:H7 strain BDMS T4169 (ATCC 700728; 4×10^{11} cfu) on d 1 of the feeding treatment, and fecal shedding of inoculated pathogens was monitored by fecal grab samples daily for 5 d (d 2 through 6). Ewes were weighed (nonfasted) and euthanized (Euthasol euthanasia solution, Delmarva Laboratories, Inc., Midlothian, VA; 0.2 mL/kg) on d 7, and intestinal contents (10 to 15 g) and tissues (10 to 20 g) from the ileum, cecum, and rectum were aseptically collected for analysis of qualitative enrichment and quantification of the inoculated strain of *E. coli* O157:H7 (as described below). Care was taken to ensure that each tissue and lumenal content sample was removed from approximately the same location on each animal. Urine was expelled from the bladder of each ewe within 1 h after euthanization, and total ergot alkaloid concentrations were determined via immunoassay (Hill et al., 2000). Changes in BW were determined by subtracting live BW before euthanization (d 7) from the BW on d 0. Figure 1 illustrates the timeline of specific activities during the experiment.

Blood serum samples were collected on d 1, 2, and 4 before the daily feeding, and on d 7 from each ewe by jugular venipuncture. Samples were allowed to clot for 24 h at 4°C and centrifuged ($1,500 \times g$ for 25 min). Serum samples were frozen (-4°C) and stored until concentrations of prolactin (Bernard et al., 1993) and cortisol (Battaglia et al., 1997; Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) were quantified in duplicate by RIA. Concentrations of NEFA were determined in duplicate by an enzymatic colorimetric proce-

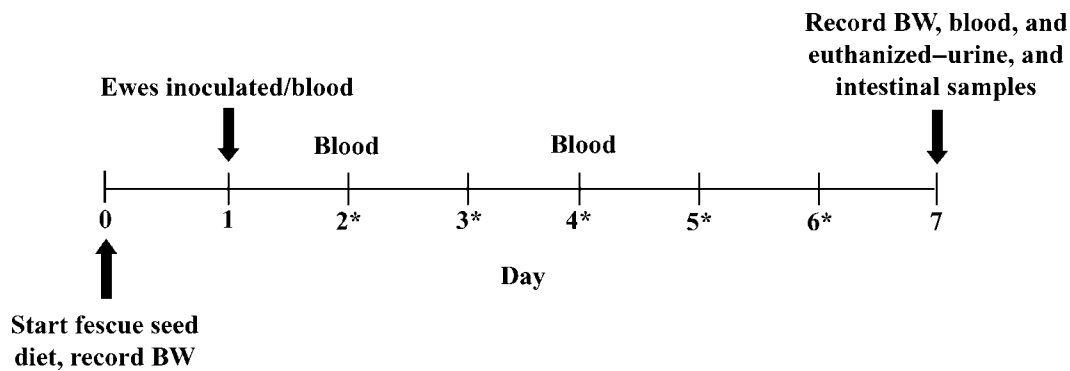


Figure 1. Timeline of events associated with the experiment. Fescue seed diets were fed from d 0 to 6; ewes were inoculated on d 1; blood samples were collected on d 1, 2, 4, and 7; fecal samples (*) were collected on d 2 through 6; and ewes were euthanized on d 7.

dure (NEFA-C, Wako Chemicals Inc., Dallas, TX) adapted for use in a 96-well microtiter plate system and expressed as microequivalents of palmitate per liter (Johnson and Peters, 1993). All samples were analyzed for each blood metabolite in single assays; the intra-assay CV were 4.0, 8.9, and 2.3% for prolactin, cortisol, and NEFA, respectively. The detection limit for each assay was 0.05 ng/mL, 0.2 μ g/dL, and 50 μ Eq/L for prolactin, cortisol, and NEFA, respectively.

Bacterial Cultures

Escherichia coli O157:H7 strain BDMS T4169 (ATCC 700728) was obtained from the American Type Culture Collection (Manassas, VA) and was cultivated in anoxic TSB medium (pH 6.7) at 37°C. This strain was made resistant to novobiocin and nalidixic acid (20 and 25 μ g/mL, respectively) and was selected via successive cultivation in TSB containing up to 20 μ g/mL of novobiocin and 25 μ g/mL of nalidixic acid (Sigma Chemical Co.; Edrington et al., 2003). Overnight cultures (1,000 mL) were harvested by centrifugation (7,500 \times g, 10 min), and the cell pellets were resuspended in TSB medium (150 mL total volume). Fecal samples were collected 3 d before dosing and screened for the presence of wild-type *E. coli* O157:H7 and generic *E. coli* resistant to novobiocin and nalidixic acid.

Bacterial Enumeration

Ten to 15 g of feces was collected from each ewe daily for 5 d (d 2 to 6). From each sample, 1 g of feces was serially diluted (10^1 - to 10^6 -fold) in sterile PBS (pH 6.5) and plated on MacConkey's agar (Difco Laboratories, Sparks, MD) that was supplemented with novobiocin (20 μ g/mL) and nalidixic acid (25 μ g/mL). Plates were incubated for 24 h at 37°C and colonies that grew on agar plates were counted directly (quantitative enumeration). To confirm the presence of inoculated *E. coli* O157:H7 qualitatively, daily fecal samples, intestinal contents, and intestinal tissue samples were incubated (24 h, 37°C) in 20 mL of GN Hajna (Difco Laboratories)

with novobiocin and naladixic acid and streaked on MacConkey's agar plates as above. Plates showing colony growth were classified as positive for the inoculated bacteria (qualitative enumeration).

Statistical Analyses

Dry matter intake, daily fecal shedding, and concentrations of prolactin, cortisol, and NEFA were analyzed by repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment, day, and their interaction were included in the model. The most appropriate covariance structure for each analysis was chosen from unstructured, compound symmetric, spatial power, and antedependence structures using Akaike's information criterion and Schwarz's Bayesian criterion (Littell et al., 2000). Kenward-Rogers' approximation was used for calculation of the df of the pooled error term. The random effect of ewe within each treatment diet (specified in the SUBJECT statement) accounted for the correlations among repeated observations of the same ewe. The most appropriate covariance structures were compound symmetric for DMI and fecal shedding, unstructured for prolactin, and antedependence for cortisol and NEFA. Concentrations of ergovaline in fescue seed and in the total diets, and the effects of treatment on BW change, urinary concentrations of total ergot alkaloid, and bacterial counts from luminal contents (quantitative) were analyzed with the MIXED procedure of SAS. A χ^2 analysis, using the FREQ procedure of SAS, was used to determine the influence of treatment on qualitative bacterial enumeration of epithelial tissue samples. Correlation analyses were used to evaluate the relationships of DMI, urinary ergot alkaloids, cortisol, and NEFA with populations of fecal *E. coli* O157:H7. Correlation coefficients were generated with the CORR procedure of SAS. When treatment differences were found, planned pairwise comparisons were evaluated using the PDIF option of SAS.

RESULTS AND DISCUSSION

Fecal grab samples collected from all ewes before inoculation failed to produce wild-type *E. coli* O157:H7

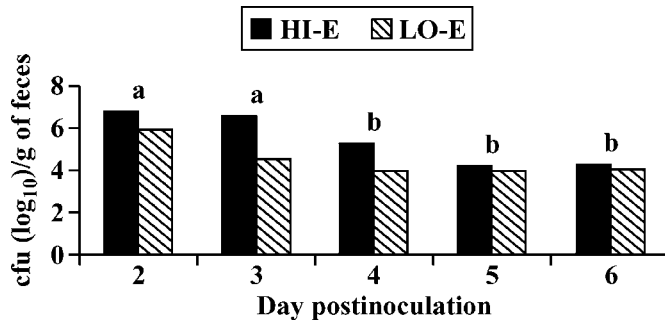


Figure 2. Fecal shedding [cfu (log₁₀)/g of feces] of *Escherichia coli* O157:H7 from ewes experimentally inoculated with *E. coli* O157:H7 and fed diets of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed. Treatment diet effect ($P = 0.06$; SE = 0.30); ^{a,b}day effect ($P < 0.001$); treatment \times day interaction ($P = 0.18$).

resistant to antibiotic selection. Shedding of antibiotic resistance-selected *E. coli* O157:H7 from experimentally inoculated ewes was influenced ($P < 0.001$) by day of collection. The prevalence of antibiotic resistance-selected *E. coli* O157:H7 peaked on d 2 and 3 postinoculation and followed a downward trend until d 6 postinoculation (Figure 2). This pattern of fecal shedding from experimentally inoculated animals was similar to previous research with experimentally inoculated sheep (Edrington et al., 2003). Populations of inoculated antibiotic resistance-selected *E. coli* O157:H7 decrease over time to a level that can be supported in the rumen, causing increased shedding of the pathogen 2 to 3 d postinoculation (T. R. Callaway, personal communication). There was no treatment diet \times day interaction for fecal shedding ($P = 0.18$); however, shedding of antibiotic resistance-selected *E. coli* O157:H7 tended ($P = 0.06$) to be increased in HI-E ewes [5.4 cfu (log₁₀)/g of feces] compared with LO-E ewes [4.5 cfu (log₁₀)/g of feces; Figure 2]. Feeding tall fescue seed diets began on d 0, and ewes were inoculated with antibiotic resistance-selected *E. coli* O157:H7 on d 1 in the current experiment. Dietary stress in ewes fed the HI-E tall fescue seed was evident by reduced DMI and a tendency to lose BW and have increased concentrations of circulating NEFA. Further, DMI was inversely correlated ($r = -0.33$; $P = 0.01$) with fecal shedding of *E. coli* O157:H7. Inoculation of calves with *E. coli* O157:H7, followed by a period of fasting, did not alter the prevalence of *E. coli* shedding (Cray et al., 1998; Harmon et al., 1999), suggesting that stressors associated with fasting in ruminants already shedding *E. coli* have minimal effects on the prevalence of *E. coli* (Harmon et al., 1999). However, shedding of *E. coli* O157:H7 from calves fasted 2 d before inoculation with *E. coli* O157:H7 shed more *E. coli* than nonfasted calves (Cray et al., 1998). The rumen environment of adequately fed animals is usually unfavorable to *E. coli*; however, undernourishment of ruminants generally reduces ruminal VFA concentrations and increases pH in the rumen,

Table 2. Luminal contents and tissue samples of gastrointestinal tract positive for *Escherichia coli* O157:H7 in ewes 7 d postinoculation with antibiotic resistance-selected *E. coli* O157:H7 and fed diets of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed

Item	HI-E	LO-E	SE
Luminal contents			
	[cfu (log ₁₀)/g of feces]		
Ileum	2.10	2.75	0.61
Cecum	2.27	3.06	0.57
Rectum	2.64	2.09	0.73
Tissue samples			
	(No. of ewes)		
Ileum ¹	6/6	4/6	—
Cecum	5/6	6/6	—
Rectum	6/6	5/6	—

¹Values in the row tend to differ ($P = 0.12$).

which can result in increased prevalence of *E. coli* (Brownlie and Grau, 1967; Rasmussen et al., 1993). Although rumen pH was not measured in the current experiment, it is unlikely that pH influenced fecal shedding of *E. coli* because consumption of toxic endophyte-infected tall fescue diets did not alter ruminal pH in steers (Harmon et al., 1991) or wethers (Aldrich et al., 1993b). In the current experiment, dietary stressors associated with consumption of HI-E tall fescue seed diets (i.e., reduced DMI) before inoculation may have caused HI-E ewes to shed more antibiotic resistance-selected *E. coli* O157:H7.

Luminal contents from the ileum, cecum, and rectum 7 d postinoculation contained similar ($P > 0.36$) populations of antibiotic resistance-selected *E. coli* O157:H7 between treatments (Table 2). Tissue samples (after a 24-h enrichment) from the cecum and rectum had a similar ($P = 0.30$) occurrence of *E. coli* O157:H7; however, ileal tissues from HI-E ewes tended ($P = 0.12$) to have an increased incidence of *E. coli* O157:H7 than did LO-E ewes (Table 2). Reduced blood flow to the intestine, decreased gut motility, or both may partially explain these differences in ileal tissues. Blood flow to the duodenum and colon was reduced in steers fed high toxic endophyte-infected tall fescue diets compared with low-endophyte diets (Rhodes et al., 1991). Ergot alkaloids reduce prolactin via dopaminergic activity (Schillo et al., 1988), and dopamine inhibits motility of the ileum in sheep (Cebrat et al., 1989). A possible decrease in motility of the ileum of HI-E ewes in the current experiment would have caused reduced particulate flow in the intestine and may have resulted in increased prevalence of antibiotic resistance-selected *E. coli* O157:H7 in ileal tissue.

Concentrations of ergovaline were greater ($P = 0.008$) for HI-E (1.63 mg/kg of DM) than LO-E (0.26 mg/kg of DM) tall fescue seed. Likewise, ergovaline was greater ($P = 0.001$) in the HI-E tall fescue seed diet compared with the LO-E tall fescue seed diet (Table 1). Approximately 70% of the more than 20 million ha of tall fescue grown in the southeastern United States is infected

Table 3. Total urinary ergot alkaloids, performance, and serum prolactin, cortisol, and NEFA concentrations of ewes experimentally inoculated with *Escherichia coli* O157:H7 and fed diets of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed for 7 d

Item	HI-E	LO-E	SE	P-value
Urinary alkaloids, ng/mg creatinine	47.8	6.2	9.4	<0.001
DMI, kg/d	0.8	1.6	0.1	<0.001
BW change, kg/d	-0.3	0.2	0.4	0.06
Prolactin, ng/mL	7.2	27.7	7.0	0.06
Cortisol, μ g/dL	3.2	4.1	0.9	0.49
NEFA, μ Eq/L	178.7	139.5	14.8	0.12

with the toxic endophyte fungus *N. coenophialum*, which produces numerous ergot alkaloids (Shelby and Dalrymple, 1987). Ergovaline is the primary ergopeptide found in endophyte-infected tall fescue (Lyons et al., 1986) and is considered to play a role in fescue toxicity (Porter, 1995); however, other ergot alkaloids also are likely involved in causing fescue toxicosis (Gadberry et al., 2003; Hill, 2005). Although threshold concentrations of ergovaline that induce fescue toxicosis are not fully established, Stamm et al. (1994) reported that the BW gain of beef steers was not affected by fescue straw containing up to 0.475 mg/kg of DM as ergovaline consumed during winter (temperature range of -3.3° to 18.9° C). Dietary concentrations of ergovaline between 0.4 and 0.8 mg/kg of DM were associated with clinical signs of fescue toxicosis in cattle and sheep at temperatures between 7.8 and 15.9° C (Tor-Agbidye et al., 2001). Fescue toxicosis is usually more detrimental to animal performance during extreme temperatures (Paterson et al., 1995). Ewes in the current experiment were housed at 23.3° C because our objectives did not include determination of environmental factors on fecal shedding of antibiotic resistance-selected *E. coli* O157:H7. Concentrations of ergovaline in the HI-E tall fescue seed diet (0.75 mg/kg) in the current experiment were similar to published values of ergovaline capable of inducing fescue toxicosis in sheep and cattle.

The urinary concentration of total ergot alkaloids was greater ($P < 0.001$) in ewes fed HI-E tall fescue seed diets compared with ewes fed LO-E tall fescue seed diets in the current experiment (Table 3). Increased concentrations of urinary ergot alkaloids are a good indication that animals have consumed endophyte-infected tall fescue (Hill et al., 2000). Accretion of urinary ergot alkaloids is usually rapid, with increased concentrations within 12 h of steers initiating endophyte-infected tall fescue grazing (Stuedemann et al., 1998). Increased urinary alkaloids were associated with reduced ADG in steers (Hill et al., 2000), and it is well established that fescue toxicosis reduces animal performance, including decreased ADG (Hoveland et al., 1983; Paterson et al., 1995).

All ewes were exposed to 14 h of light 2 wk before initiation (d 0) and during the experiment. Concentrations of prolactin were similar ($P = 0.26$) for HI-E and

LO-E ewes on d 0 and averaged 17.5 ± 4.9 ng/mL. Concentrations of prolactin were not affected ($P = 0.26$) by day or by treatment diet \times day interaction ($P = 0.36$) in the current experiment; however, the main effect of the treatment diet tended ($P = 0.06$) to influence concentrations of prolactin. Ewes fed HI-E tall fescue seed diets tended to have reduced concentrations of prolactin compared with LO-E ewes, providing further evidence of fescue toxicity in HI-E ewes (Table 3). Ergot alkaloids decrease concentrations of prolactin via dopaminergic activity (Schillo et al., 1988; Porter and Thompson, 1992); thus, prolactin is frequently used as a physiological indicator of fescue toxicosis in ruminants (Aiken et al., 2006). In the current experiment, the increase in urinary alkaloids and decrease in prolactin in ewes consuming HI-E tall fescue provided confirmation that ergot alkaloids in HI-E tall fescue seed diets were adequate to induce physiological changes associated with fescue toxicosis.

Ewes fed HI-E tall fescue seed had lower ($P < 0.001$) DMI than did LO-E ewes; consequently, HI-E ewes tended ($P = 0.06$) to lose and LO-E ewes tended to gain BW during the 7 d (Table 3). Dry matter intake is usually reduced in ruminants consuming endophyte-infected tall fescue (Paterson et al., 1995; Gadberry et al., 2003; Parish et al., 2003); thus, BW gain is reduced in cattle (Nihsen et al., 2004; Looper et al., 2006) and sheep (Gadberry et al., 2003; Parish et al., 2003). Controlled intake studies that mitigated differences in DMI between toxic endophyte-infected and endophyte-free diets have shown that BW gains were reduced in cattle consuming endophyte-infected tall fescue diets (Fiorito et al., 1991; Mizinga et al., 1992; Jones et al., 2003). Other factors such as decreased digestibility (Humphry et al., 2002) and gut motility (Cebat et al., 1989) may be responsible for the decreased performance of ruminants consuming endophyte-infected tall fescue diets. We acknowledge that reduction of DMI was confounded with consumption of HI-E diets; however, the current experiment was not designed to delineate the independent effects of alkaloids, DMI, or both.

Concentrations of NEFA tended ($P = 0.12$) to be greater in HI-E ewes than LO-E ewes (Table 3). Further, concentrations of NEFA were inversely correlated ($r = -0.43$; $P = 0.002$) with DMI. Nonesterified fatty

acids are an indicator of the nutritional status of ruminants, and NEFA are released into circulation during periods of chronic nutrient restriction when adipose tissue is catabolized (Bossis et al., 1999). Recently, Ciccioli et al. (2003) found that NEFA in cows fed to gain 0.9 kg of BW/d was increased within 7 d when changed to a diet to gain 0.45 kg of BW/d. Reduced DMI in ewes fed HI-E tall fescue seed diets caused subsequent BW loss and a tendency for concentrations of NEFA to be increased compared with LO-E ewes. These data, along with increased urinary ergot alkaloids and a tendency for decreased prolactin, confirm that ewes fed HI-E tall fescue seed diets for 7 d were experiencing fescue toxicosis.

Concentrations of cortisol were not affected by treatment diet ($P = 0.49$; Table 3), day ($P = 0.24$), or their interaction ($P = 0.69$); the overall mean concentration of cortisol was $3.7 \pm 0.9 \mu\text{g/dL}$. Cortisol has been associated with stress in ruminants (Gwazdauskas et al., 1972), and cortisol was increased in steers and cows 3 to 4 h after infusion of the ergot alkaloid ergotamine (Browning et al., 1998). However, when samples were collected daily, concentrations of cortisol in heifers (Aldrich et al., 1993a) and lambs (Fiorito et al., 1991) adapted to toxic endophyte-infected tall fescue diets for 10 to 14 d were similar to cortisol in animals consuming endophyte-free tall fescue diets, suggesting that ruminants may become acclimated to the toxic effects of endophyte-infected tall fescue. Concentrations of cortisol were similar between ewes fed HI-E or LO-E tall fescue seed diets in the current experiment and may not be a good indicator of stress induced by HI-E tall fescue for 7 d.

Short-term (7 d) consumption of HI-E tall fescue seed decreased DMI, increased urinary concentrations of total ergot alkaloids, and tended to reduce BW and circulating concentrations of prolactin in ewes experimentally inoculated with *E. coli* O157:H7. Ewes fed HI-E tall fescue seed diets tended to shed more antibiotic resistance-selected *E. coli* O157:H7 in their feces and tended to have an increased incidence of antibiotic resistance-selected *E. coli* O157:H7 in their ileal tissues than did LO-E ewes.

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